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A PART OF A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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By
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ACTION OF WATER MOLECULES
IN THE ISOLATED
FROG HEART

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THE ACTION OF WATER MOCCASIN VENOM ON THE ISOLATED FROG HEART

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The action of various snake venoms on isolated frogs hearts has been studied by many investigators. Cobra venom has been so studied by Elliott (1901), Gunn (1912), Cushney and Yagi (1918), Gunn and Heathcote (1921), Meurling (1935), and others. Magenta (1922) studied the effects produced by venoms from fifteen species of poisonous snakes, by the Straub method. Unfortunately his results are published in such abbreviated form that the effects of individual venoms cannot be determined.

Among the venoms Magenta used was that of the water moccasin, *Agkistrodon piscivorus* Lacépède. The action of the venom of this species of snake has also been studied by Essex and Markowitz (1930), and by Essex (1932), using hearts of rabbits. When a Ringer-Locke perfusion fluid to which dried venom was added to make a final dilution of 1:250,000 to 1:500,000 was used, the hearts were incapacitated in ten to fifteen minutes, indicating a much greater cardiac toxicity than is possessed by the venom of the timber rattlesnake, *Crotalus horridus*. In view of the small amount of study of the water moccasin venom, the author determined to investigate the venom, especially because of the frequent statement that this venom is essentially similar to that of the North American rattlesnakes, e.g., Noguchi (1909), Essex and Markowitz (1930), Essex (1932).

In all experiments, isolated hearts of spring or summer frogs were used. A modified Straub method, "constant drip," was necessary. The modifications were: 1, arrangement of the reservoir containing the perfusion fluid so that a constant stream of fluid ran into the cannula; 2, a cotton wick at the upper end of the cannula, which prevented formation of a

large meniscus, with consequent alteration of the diastolic filling; it also distributed the overflow evenly over the exterior of the heart. The rate of flow of the perfusion fluid was such that one or two drops were expelled at each systole.

The venom was collected in the latter part of the summer of 1938, by the well-known method of "milking." The collected venom was dried under an electric fan, ground in a mortar and the various batches mixed to form the stock dry venom. The stock represented ten milkings from twenty-eight snakes. Mitchell and Reichert (1886), and Macht (1933 and 1937) have shown that any alteration in such dried venoms is in the direction of some loss in potency, if there be any change.

The perfusion fluid used in all experiments was Ringer solution of the following composition, expressed in millimoles per liter: NaCl 112.20, KCl 1.88, CaCl_2 1.04, NaHCO_3 2.38. To this was added enough weighed dry venom to give the desired dilution.

The contractions of the hearts were recorded on a kymograph by means of a light heart lever, fastened to the tip of the ventricle by a fine silk thread. For each dilution used eight¹ hearts were run. In the control group of eight hearts, one failed completely in 180 minutes. This duration was considered as maximum in all subsequent experiments. In experiments with venom, the hearts were run in the Ringer solution until they had become physiologically constant; at this time the cannula was filled with the Ringer-venom solution and the drip started.

Each record was measured for amplitude of contraction. For each, the initial amplitude was arbitrarily considered as ten centimeters, an approximation of the actual average amplitude. These results were averaged for each experimental group, i.e., controls, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 venom-Ringer solutions. The results are shown as figure 1.

The control hearts run with Ringer solution showed no stimulation and a decrease in amplitude of 43 per cent in 180 minutes; the hearts subjected to 1:1,000 venom in Ringer showed practical standstill in 18 minutes and no stimulation throughout the experiment. When venom was used in a concentration of 1:10,000 there was a brief period of increased amplitude (maximum: 43 per cent; duration: 7 minutes) succeeded by rapid decrease to zero in 24 minutes. The solution of venom 1:100,000 produced a maximum increase of 8 per cent; the duration of the stimulating action was 17 minutes absolute or 43 minutes when compared with the controls. Hearts run with 1:1,000,000 venom-Ringer solution showed an average maximum stimulation of 11.4 per cent and remained more effective than the controls throughout the entire 180 minutes.

¹ The group run in 1:100,000 venom consisted of seven hearts.

The effects on heart rate of the Ringer solution and the various venom solutions are shown in figure 2. The rate of beating of the controls remained rather uniform, i.e., O. Frank's law was applicable, the changes

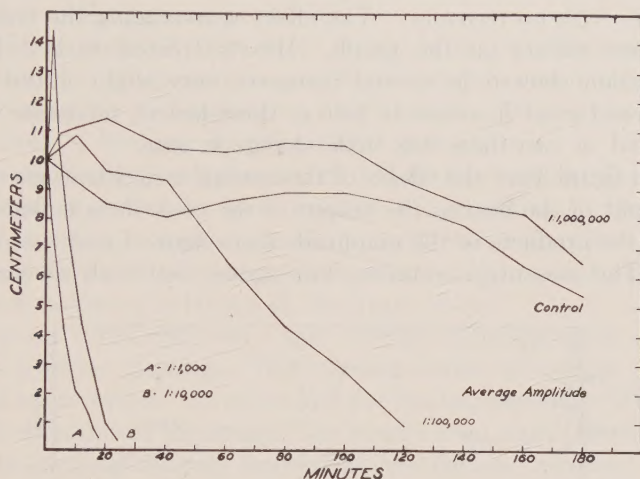


Fig. 1. Average contraction amplitudes of control hearts and those subjected to venom-Ringer solution.

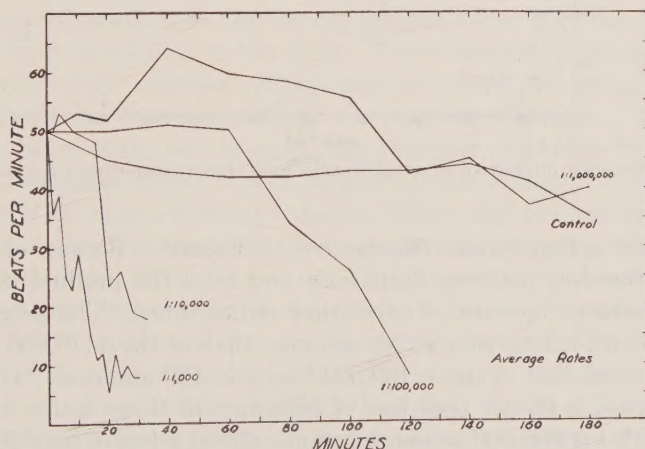


Fig. 2. Average rates of control hearts and those subjected to venom-Ringer solution.

in rate being in general inversely proportional to the changes in amplitude. In 180 minutes the rate of the controls decreased 35 per cent. Marked slowing or complete stoppage resulted from the application of 1:1,000 venom solutions; partial recovery followed. With 1:10,000 venom solutions

there was first marked slowing, followed by a slight increase in rate; this in turn gave way to rapid failure of contraction. The original slowing could be abolished by atropine. In 1:100,000 venom solutions, three of the hearts showed initial slowing, three initial stimulation, and one showed rapid failure with no recovery. The effect of averaging the results is to obscure these effects on the graph. Hearts treated with 1:1,000,000 venom solution showed in several instances very slight initial slowing. There followed great increases in rate in three hearts, moderate increases in three, and in two there was little change in rate.

Shown in figure 3 are the effects of the various concentrations of venom on the output of the hearts; the output of the controls is included. The curves are the products of the amplitude from figure 1 and the rate from figure 2. The measure is relative, but agrees well with several experi-

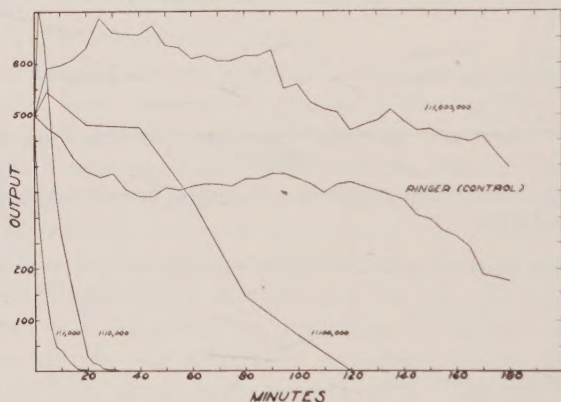


Fig. 3. Average output of control hearts and those subjected to venom-Ringer solution.

ments where actual volume changes were measured. Because of the reciprocal relationship between amplitude and rate, the product of the two is a more reliable measure of effect than either alone. The output of the 1:1,000 hearts fell to zero in 20 minutes; that of the 1:10,000 hearts in 32 minutes and that of the 1:100,000 hearts in 120 minutes. The control hearts showed a 65 per cent loss of efficiency in three hours; the hearts treated with 1:1,000,000 venom solution suffered a loss of only 20 per cent in the same time. The first two solutions were severely cardiotoxic; the 1:1,000,000 solution was beneficial; the 1:100,000 solution produced stimulation, followed by rapid failure.

The order of failure in the hearts was first the ventricle, then the atria, last the sinus venosus. The ventricle stopped in complete contraction in most of the hearts. In those treated with high dilutions of venom the

contraction was sometimes incomplete; mechanical stimulation induced complete contraction. The muscle became whitish and opaque, with a "cooked" appearance. The atria failed in wide dilatation. They were also opaque. The sinus venosus remained clear and beat for some time after failure of the atria and ventricle.

Dissociation occurred only rarely except in the terminal stage of ventricular activity, where complete ventricular failure was imminent. This is in contradiction to Magenta's (1922) results. When dissociation did occur, it was of the type of dissociation with capture.

Extra systoles were rather common. Muscular irregularities frequently occurred, accompanied by local regions of contracture in the ventricle. These effects disappeared as the elasticity of the myocardium decreased, a constant effect with all venom solutions except the weakest, as shown by progressive failure of rebound of the heart lever.

The venom acted upon the vagal endings or the ganglion cells of the heart to produce slowing. This slowing could be almost completely abolished by atropine. In several of the hearts, especially those treated with high dilutions of the venom, the release from vagal effects was quite sudden; in one case the rate doubled in less than one minute. The probable site of action is the ganglion cells, which are first stimulated, then depressed. This is in accordance with the stimulation and subsequent depression of the myocardium seen above.

An outstanding effect of the venom solutions was the tremendous increase in the permeability of the atria. These became so permeable that the "constant drip" method was necessary to keep the Straub cannula filled. Obviously the endocardium was severely damaged. There appeared to be no fluid loss through the ventricle, probably because the much heavier, more dense muscle prevented fluid escape.

Because of the many obvious muscular effects and the constant dialyzing action of the "constant drip," the author believes the results to be due to direct action of the venom on the myocardium, endocardium, and intrinsic ganglia, rather than to the action of lysolecithin and histamine produced by the action of the venom on the tissues. This view is in agreement with that of Essex (1932) and receives correlative support from the work on rattlesnake (*Crotalus horridus*) venom of Dunn (1934); Essex and Markowitz (1930b); Dragstedt, Mead, and Eyer (1938); and from the demonstration of such direct effects of snake venoms on protoplasm by Lepow (1938) and the importance of the change of lecithin into lysolecithin on the properties of surface films as shown by Hughes (1935). The hypothesis that the effects of the venom are caused by the lysolecithin and histamine produced, as stated by Belfanti (1925), Magenta (1922), Houssay (1930), and Houssay, Negrete, and Mazzocco (1933)

seems unnecessarily complex. This aspect of the problem is well reviewed by Kellaway (1939). The author believes the venom acts directly and that the lysolecithin and histamine are by-products of such direct action, rather than being the causative agents of the tissue changes.

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SUMMARY

The venom of the water moccasin, *Agkistrodon piscivorus* Lacépède, acts directly on the endocardium, myocardium and intrinsic ganglia of the isolated frog heart.

In 1:1,000 concentration, the venom has a depressant action on the heart; in 1:10,000 and 1:100,000 concentrations there is preliminary stimulation, followed by failure.

Concentrations of 1:1,000,000 are stimulating; the hearts exceeded the efficiency of the controls throughout the experimental period.

The ventricle failed first; stoppage was usually in complete contraction, although with weak venom solutions it might be in partial contraction. There were muscular irregularities and contractures with all but the weakest solutions.

The atria were rendered highly permeable, permitting rapid escape of fluid through their walls. Failure was in extreme dilatation, occurring after that of the ventricle.

The sinus venosus appeared to be quite refractory to the venom action and failed last.

Dissociation rarely occurred, except in terminal period of ventricular action, where it presaged complete failure in short order.

Stronger venom solutions slow the heart initially by action on the vagus mechanism, probably the ganglion cells. This slowing can be almost completely abolished by atropine.

After failure both the atria and ventricle were opaque and whitish; they looked "cooked."

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